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DEXAMETHASONE-INDUCED T-LYMPHOCYTE APOPTOSIS IN DIFFERENT LYMPHOID ORGANS

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ABSTRACT

dexamethasone (DEXA) influence on T-lymphocytes in central (thymus) and peripheral (spleen, lymphatic nodes) immune organs. For that reason therapeutic doses of DEXA were used followed by histological, histochemical (TUNEL) as well as computerized histomorphometrical investigations.

In the study 36 young adult Wistar rats performed. 1 – 7 days after 3 days injection of DEXA (total dose 1,2 mg/rat i.p.) the material was taken for futher investigations. First days after DEXA administration in therapeutic doses the number of apoptotic cells was considerably increased in the cortical part of thymus. No significant changes were in rest of thymus as well as in peripheral immune organs. 7 days after DEXA-induced injury the number of apoptotic cells had decreased almost to the normal level.

Our investigations conclude that the most sensitive for the dexamethasone-induced T-lymphocyte apoptosis is cortex of thymus while the changes in medullary area of thymus and peripheral immune organs – spleen and perithymic lymphatic nodes- are less significant. Week after drug cessation the apoptotic changes are almost at normal level in both types of lymphoid organs.

Key words: *apoptosis, dexamethasone, thymus, spleen, lymphatic nodes*

INTRODUCTION

Apoptosis is an active process of cellular self-destruction, called programmed cell death, that is implicated in both normal and pathologic tissue changes (1). Apoptosis is frequently present in tumours and tissues treated with a variety of toxic stimuli (2). As changes in normal apoptotic processes may cause diseases and disorders in homeostasis the investigations of apoptosis have increased recently (3–5).

Increase in apoptosis in immune organs is involved in enhancement of the immune response**.** It is widely known that central immune organ thymus is vulnerable to radiation and immunotoxic chemicals (6–8). Still there are no comparative studies on apoptosis of central and peripheral immune organs – comparison of apoptosis on T-lymphocytes in different lymphoid organs.

Thymocyte apoptosis plays a physiological role in the T-cell selection in the thymus (9) Apoptosis occurs in the cells of the thymus cortex where it is accelerated by glycocorticoid hormones (4). Probably therefore for the study of thymocyte apoptosis dexamethasone-model is one of the best characterised experimental models (10–11).

The aim of the present study was to compare the apoptotic changes in different immune organs using synthetic glucocorticoid dexamethasone in therapeutic dosages.

MATERIAL AND METHODS

36 male young adult Wistar rats with a weight of 200–220 g were investigated on 1 to 7 days after dexamethasone (DEXA) administration. The guidelines for the care and use of the animals were approved by the Ethical Committee of the University of Tartu. The rats were housed in an animal room, controlled at temperature, 22±2ºC; humidity, 55±15%; ventilation (all-fresh-air system); 12 h light/dark cycle and fed a certified diet "Dimela" (Finland R-70 and R-34) and water ad libidum. Animals were acclimated for 7 days before dosing.

The rats were injected with DEXA 1,2 mg/rat i.p. on the 1st, 4th and 7th days and sacrified 1, 3, 5 and 7 days after last injection. The animals were euthanized by decapitation under anesthesia with i.m. injection of ketamine 50 mg/kg b. w. and diazepam 5 mg/kg. The lymphoid organs – thymus, perithymic lymphatic nodes (mediastinal lymph nodes) and spleen, were carefully removed. One-half of each organ was frozen in

liguid nitrogen and stored at –80ºC until used for histochemistry (TUNEL). The remaining parts were used for routine histology as well as for lymphocyte detecting by histomorphometry: the lymphoid organs were fixed in 10% buffered formalin, and embedded in paraffin, thereafter 7 μm thick sections were stained with haematoxylin – eosin, Heidenhain's iron haematoxylin and Feulgen. For better understanding of apoptotic changes, mostly occurring in the cortical and medullary cells in the thymus, computerized morphometry was used: the pictures of slices stained for routine histology were photographed by a light-microscope Olympus BX-50, saved electronically and the further process was performed with the computer program Adobe Photoshop 5.0 under a simultaneous visual control of light-microscopy (11). The areas of thymolymphocytes were observed and analysed with the help of Adobe Photoshop selecting different colours (apoptotic/non-apoptotic): the painted areas were summarised in pixels and the proportions of different cells were calculated in percents.

Statistical analysis was performed using one sample t-test and the unpaired t-test (GraphPad Quick Calcs: Analyze continous data) at the level of significance p less than 0,05 ($p<0,05$) with the Newman-Keuls multiple comparison test.

Computerized histomorphometry is widely used in the quantitative analysis (12–15).

For histochemistry frozen tissue sections were stained for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) with a standard in situ Cell Death Detection Kit – POD (Roche Molecular Diagnostics, U.S.A.), according to the manufacture's guidelines (16–19). The apoptotic cells were detected by TUNEL; each experiment set up by TUNEL assay without terminal transferase served as negative control.

RESULTS

The apoptotic cells (Ao) were detected in rat thymus, spleen and perithymic lymphoid tissue(mediastinal lymph nodes). For better understanding of the apoptotic changes in thymus, the TUNEL-positive cells were detected separately in cortical and medullary part of thymus (Fig. 1).

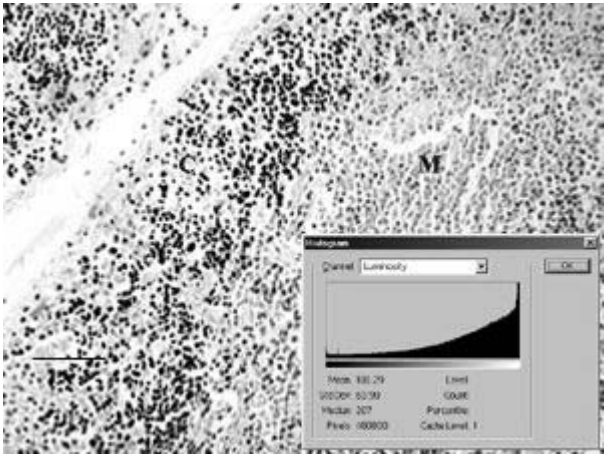

Figure 1. Group of apoptotic cells in outer cortex (C) of thymus lobe; medullary area (M) remains lightly stained. Iron haematoxylin. Bar: 100 μm.

During the first days after DEXA administration the number of Ao cells detected by Photoshop analysis and TUNEL-positive cells were the largest in the cortical part of thymus (Table 1). In compare to the rest of thymus and secondary lymphoid organs the remarkable increase of the percentage of Ao cells and amount of TUNEL-positive cells in deep cortex were noted (Fig. 2).

The high number of Ao cells and TUNEL-positive cells in cortical part of thymus continued up to 5^{th} days whereas the changes in medullary area of thymus as well as in spleen and perithymic lymphatic nodes remained almost at control levels. In spleen the highest increase of Ao cells was noted during first days after injury in germinal centres of splenic nodules and around central artery (Fig. 3). Among the different parts of lymphatic nodes the highest increase of Ao cells was in paracortex compared to cortical and medullar parts (Fig. 4).

7 days after cessation of DEXA administration the expression of apoptosis was at almost normal levels (Table 1).

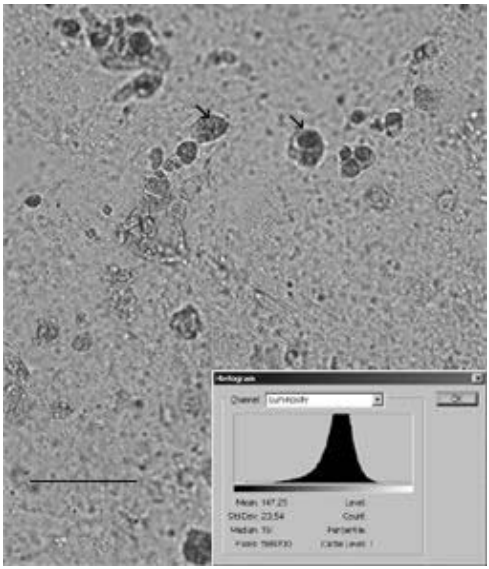

Figure 2. The large number of the apoptotic TUNEL-positive cells (indicated by arrows) in thymus cortex 1 day after the i.p. DEXA injection in rat. Histogram on the whole field (769600) pixels. Bar: 100 μm.

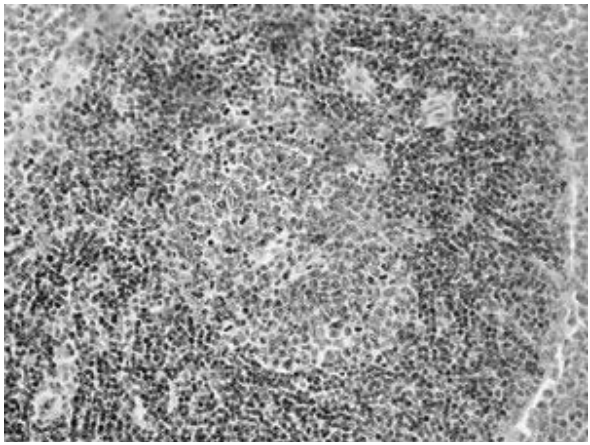

Figure 3. Spleen 1st day after injury: destruction of follicles, depletion of parenchyma and distribution of the apoptotic cells mainly in outer part of splenic nodule. Haematoxylin-eosin. Bar: 100 μm.

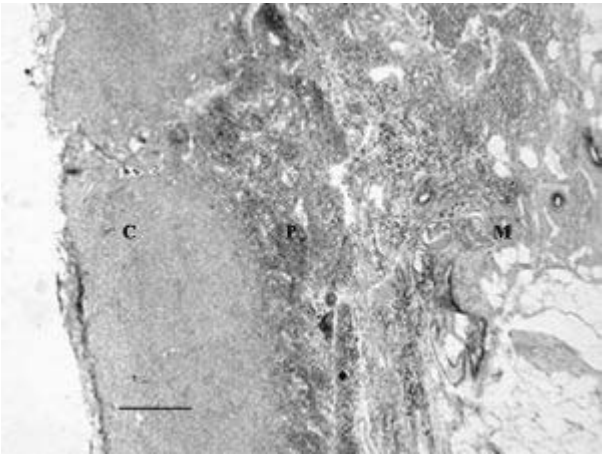

Figure 4. Distribution of apoptotic cells in cortical (C), paracortical (P) and medullary (M) area of lymph node 1 day after the i.p. DEXA injection in rat. Increase of apoptotic cells in paracortical area. Haematoxylin-eosin. Bar: 500 μm.

Table 1. Areas of apoptotic cells (APC) in rat thymus 1–7 days after the DEXA injection 1,2 mg/rat i.p. (percentage of total cell area ±SEM). Computer field in use: 16,7%; 118306 pixels (=100%)

Days after DEXA injection	**Cortical area (inner cortex)**	**Medulla**
1	48,0±1,9*	2,3±0,3
3	28,1±1,2*	1,9±0,2
5	13,4±0,5	2,4±0,3
7	9,2±0,7	1,6±0,1
Control 6,3±0,8	1,2±0,1	

*differences between the values of this group are significant ($p<0,05$)

Our findings suggest that for the dexamethasone induced apoptosis the most sensitive in compare to different lymphoid organs are T-lymphocytes locating in the cortical part of thymus, especially in deep cortex. Week after the drug cessation the apoptotic changes become almost at normal level in both types of immune organs.

DISCUSSION

Apoptosis is a genetically mediated programmed cell destruction where cells die, shrink, and disintegrate in the absence of any reactive inflammation. Autoreactive T- and B-lymphocytes are also eliminated by apoptosis, ensuring homeostasis for organism. Dearangements of the process of apoptosis can lead to deformities and tumors (20). Apoptosis is frequently present in tissues treated with a variety of toxic stimuli (2).

Endogenous glucocorticoids (GC) take part in homeostasis. Overflow of exogenous GC have caused appearance of thymic atrophy, inhibition of mitotic activity of cortical thymolymphocytes and fall the number of cells. Synthetic GC dexamethasone (DEXA) in therapeutic dose caused thymus atrophy enhancing the number of Ao cells, especially in the cortical area (2,21).

In our experiments the synthetic glycocorticoid dexamethasone in therapeutic doses was used to compare the apoptotic changes in primary and secondary lymphoid organs. The countings of Ao cells and histochemistry by TUNEL reaction after DEXA administration showed the clear difference of apoptotic changes in different immune organs, moreover the different localization of apoptosis of a particular organ. For example one day after DEXA injection the percentage of Ao cells of total cell area in rat thymus were in cortical area 48,0±1,9 and in medullary area 2,3±0,3 compared with control values 6,3±0,8 and 1,2±0,1 respectively.

Ao cell values are more high compared to TUNEL-positive cells, because the Ao cell group consists besides of darkly staining proper apoptotic cells also of some necrotic and mitotic cells, which are non-differentiated in photoshop-based image analysis. Still, the number of Ao cells, markers of lymphocyte proliferative rate are widely used (22–27).

TUNEL-positive cell specifity varies with tissue and degree of injury. After therapeutic doses of DEXA TUNEL-sensitivity of thymolymphocytes is 100% in correlation with histology. TUNEL specifity after predominantly necrotic injury do not exceed 70% of detected apoptotic cells (28). In mouse kidney the apoptotic signal detected with TUNEL assay may be false-positive (29).

The TUNEL-positive cells are apoptotic cells *in situ*, because the chromosomal nick ends are directly labelled (7,10). Therefore our indirect detection of Ao cells by computerized histomorphometry

simultanously with TUNEL assessment, is quite correct for the measurement of apoptotic thymolymphocytes.

Our experiments showed that after DEXA administration the most sensitive were T-lymphocytes in central immune organ thymus. Sensitivity of thymolymphocytes to DEXA-induced apoptosis varies with exo- and endogeneous factors. DEXA activated thymocyte apoptosis requires a sequence of events including interactions with the GC receptor, phosphatidylinositol-specific phospholipase C, acidic sphingomyelinase activation (30).

The initiation of apoptosis needs triggering of primed cells into apoptosis itself – stimuli that trigger primed cells will not initiate apoptosis in unprimed cells. The immature T-cells of the thymus cortex are primed for apoptosis and receptor occupancy triggers apoptosis in them. In contrast, the mature T-cell is not primed and responds to the same ligand by initiating cell replication instead of apoptosis in medullary area of thymus (1). Probably the same reason occurs also in peripheral immune organs. More investigations (genetic, electron-microscopic etc.) for solving the questions are required in future.

Our investigations conclude that the first days after DEXA-administration in therapeutic doses the most sensitive for the dexamethasone-induced T-lymphocyte apoptosis is the cortical area of thymus while the changes in medullary area of thymus as well as in peripheral immune organs are not remarkable. In spleen the most sensitive parts were the splenic nodules and in lymph nodes the paracortical area.

The apoptotic changes caused by synthetic glycocorticoid dexamethasone are reversible. 5–7 days after drug cessation the apoptotic changes become almost at normal(control) level in both types of lymphoid organs.

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